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Soil persistence and biodiversity of ericoid mycorrhizal fungi in the absence of the host plant in a Mediterranean ecosystem

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Abstract The occurrence of suitable mycorrhizal inocula may be an important factor affecting the dynamics of plant communities. We investigated the persistence and diversity of ericoid mycorrhizal fungi in the soil of a mature *Quercus ilex* forest where ericaceous hosts were absent. *Erica arborea* was used as a bait plant and results were compared to soil samples from experimental plots where cuttings had allowed reappearance of this ericaceous species. Fungal endophytes were isolated and tested in mycorrhiza resynthesis trials. Sterile mycorrhizal endophytes were assigned to morphotypes whose consistency was confirmed by ITS-RFLP. The ITS region of a representative of each morphotype was sequenced. BLAST searches and Neighbour-Joining analysis indicated taxonomic affinities with different classes within Ascomycota. Our results indicate that ericoid mycorrhizal fungi persist and maintain mycorrhizal ability in habitats lacking the ericaceous host. Their persistence could favour the establishment of *E. arborea* seedlings in pure *Q. ilex* forests after disturbance phenomena.

Keywords Plant succession · Mediterranean ecosystem · Post-cutting · *Erica arborea* · *Quercus ilex*

Introduction

Mediterranean ecosystems develop in temperate-warm climates with rainfall ranging from 300 to 1,000 mm/year and with characteristic summer dryness. These environments can feature a high diversity of plants and their associated mycorrhizal types: sclerophyllous and evergreen shrubs and small trees bearing ericoid, arbuscular, arbutoid and ectomycorrhiza coexist and may be of equal sizes and dominance (Allen 1991). If left undisturbed, such co-occurrence of mycorrhizal types can develop into dominance of ectomycorrhiza in the later stages of the succession that leads to mature forests. This is, for example, the case with *Quercion ilicis* Br.-Bl., the pure *Quercus ilex* L. climax woodland commonly found in Mediterranean areas of Northern Italy. This plant community is characterized by an extremely reduced understorey vegetation, due to the disappearance of several plant species. The disappearance of mycorrhizal hosts poses the intriguing question of the potential persistence in the soil of the associated mycorrhizal partners, especially for those mycorrhizal fungi that show a relatively high degree of host specificity, such as ericoid fungi. Ericoid fungi are indeed mostly known to associate with a few plant genera within the Ericales (Straker 1996), but early observations suggest that ericoid mycorrhizal fungi occur in soils which have not been colonized by ericaceous plants (Pearson and Read 1973).

Human activities and fire play a key role in shaping Mediterranean vegetation. In the climax woodland, such disturbances open the way for recolonization by pioneer plant species, especially Ericaceae, thus initiating secondary successions. Under these conditions, survival of ericoid mycorrhizal fungi in the mature woodland, where the host plant is absent, could be an important determinant of plant diversity and dynamics.

The aim of this work was, therefore, to investigate the persistence of ericoid fungi in a Mediterranean *Quercus ilex* climax forest where the host plant *Erica arborea*, a typical ericaceous Mediterranean plant, was absent. The inoculum potential and diversity of ericoid mycorrhizal fungi were assessed and compared to samples taken from post-cutting clearings where the pioneer shrub species *E. arborea* had re-established.

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Materials and methods

Sampling

The Mediterranean *Quercus ilex* forest was located at Borgio Verezzi, Ligury, Italy. The areas investigated were situated: (1) within a pure woodland where *Q. ilex* establishment had caused the disappearance of most other species, including *Erica arborea* ("Q" areas); (2) in clearings where cutting had allowed reappearance of the shrub community represented by *E. arborea* L., *Pistacia lentiscus* L., *Cistus monspelliensis* L., *Arbutus unedo* L., *Ostrya carpinifolia* Scop., *Q. ilex* L. and *Quercus pubescens* Willd. ("E" areas). Fungi associated with *E. arborea* and *Q. ilex* roots in the E areas had been analysed in a previous investigation (Bergero et al. 2000). No specific data are available for the Q areas as to time elapsed since the disappearance of *E. arborea*. Literature data suggest that at least 10–20 years are needed for mature *Q. ilex* forest to re-establish after disturbance (Pignatti 1990). Soil samples were taken in the thickest part of the forest in the Q areas under mature tree specimens.

In May 1997, five plots from the Q areas and four plots from the E areas close to healthy *E. arborea* plants were selected. After litter removal, three soil samples (approximately 2 kg each) were collected from each plot (approximately 1–2 m from *Q. ilex* individuals), placed in plastic bags and processed in the laboratory within 2 weeks of sampling.

Assessment of ericoid mycorrhizal infection and isolation of fungal associates

Inoculum potential and diversity of ericoid fungi in soils was assessed using *E. arborea* as bait plant. Three 2-month-old axenic *E. arborea* seedlings were obtained from surface-sterilized seeds according to Bergero et al. (2000) and were planted in 12-cm-diameter pots (one pot/soil sample). Pots were placed in a growth chamber (16 h/8 h day/night cycle, 22°C day/18°C night) and were watered twice a week with a half-strength Long Ashton solution (Hewitt 1966).

After 5 months, plants were harvested and processed to assess ericoid mycorrhization and to isolate fungal endophytes. Root segments (five approximately 3-cm-long segments/plant) were thoroughly cleaned of soil debris under a stereomicroscope, washed under a continuous stream of tap water for 24 h, surface sterilized in 30% H_2O_2 for 10 s, rinsed twice and gently homogenized. The cell suspension was plated on 2% malt agar supplemented with 20 mg/l streptomycin. Sporulating fungal cultures were identified and sterile mycelia were assigned to distinct morphotypes.

Mycorrhiza resynthesis trials

Representatives from each sterile morphotype were tested for their ability to produce ericoid mycorrhiza on axenic *E. arborea* seedlings, according to the protocol of Pearson and Read (1973). Agar plugs from fungal cultures growing on 2% malt extract agar and axenic *E. arborea* seedlings growing on 0.1× Murashige and Skoog agar (Murashige and Skoog 1965) were transferred to Magenta vessels (Sigma) containing a bottom-layer of water agar and a top-layer of sterilized soil. About 3 months later, hair roots were examined by light microscopy and checked for the presence of a typical ericoid mycorrhizal phenotype. Mycorrhizal infection was evaluated by counting the percentage of coil-harbouring epidermal root cells.

Molecular analyses

ITS/RFLP analysis was used to compare isolates within and among sterile mycorrhizal morphotypes. DNA was extracted from 30–50 mg of fresh mycelium according to Perotto et al. (1996). PCR amplification of the ITS region was carried out with the primer pair ITS1/ITS4 (White et al. 1990) and the amplified fragment was digested with three restriction enzymes (*Hinf*I, *Mbo*I, *Hae*III).

The ITS region (ITS1–5.8S-ITS2) was sequenced for a representative isolate of mycorrhizal morphotypes Sd1, Sd3, Sm1, Sm2, Sm3, Sm5 and Sm8. ITS sequences for morphotypes Sd2 and Sd9 had been obtained in previous work (deposited in GenBank under the accession numbers AF269067 and AF269068, Bergero et al. 2000). The PCR products obtained by amplification with ITS1 and ITS4 primers (White et al. 1990) were gel purified using a QiaexII Gel Extraction Kit (Qiagen) and sequenced by GenomExpress (Grenoble, France). Sequences were submitted to GenBank. Searches for the most similar sequences in sequence databases were performed by the NCBI BLAST search algorithm (Altschul et al. 1997). Sequence alignments were created with the ClustalX v. 1.8 programme (Thompson et al. 1997) and adjusted manually with GeneDoc to optimize alignment. Identity percentage between sequences was calculated with an on-line version of the ClustalW programme (Thompson et al. 1994; http://pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalwan.html). Sequences of morphotypes Sd1, Sd9, Sm5, Sm8, Sm1, Sm2 and Sm3 could be aligned with some confidence and, therefore, were subjected along with other alignable sequences from BLAST searches to neighbour-joining (NJ) analyses. NJ analyses were performed with TREECON version 3.0 (Van de Peer and De Wachter 1993) using Kimura 2-parameter distances and the automatic root location option. Robustness of the internal branches was assayed by bootstrap analysis (1000 runs). Taxonomic assignment for teleomorphic taxa follows Kirk et al. (2001).

Results

Fungal isolation and mycorrhization trials

Bait plants grown in three different soil samples from either E or Q plots were successfully mycorrhizal after 5 months. Comparable mycorrhization levels were found for soil samples collected from the same plot. Similarly, no significant differences were found in the percentage of infection of E and Q plots.

During the isolation procedure, 641 and 762 fungal isolates were obtained from the roots of bait plants grown in E and Q soils, respectively. Most were sterile mycelia (89% of isolates obtained from E and 87% from Q soils) and were assigned to distinct morphotypes based on colony and hyphal morphology. Representatives of each morphotype were tested for their ability to form ericoid mycorrhizal coils in axenic conditions. Species of *Oidiodendron* were also obtained from both kinds of soils. *Oidiodendron* spp. are commonly described as ericoid mycorrhizal endophytes (Couture et al. 1983; Dalpé 1991; Hambleton and Currah 1997), but they were excluded from resynthesis trials due to their poor ability to produce in vitro mycorrhizal infection on axenic *E. arborea* seedlings (Bergero et al. 2000). In resynthesis trials, several sterile isolates belonging to ten morphotypes produced coils in the epidermal root cells of *E. arborea*, thus displaying the typical ericoid mycorrhizal phenotype (Table 1). Eight mycorrhizal morphotypes were recovered from the E soil and accounted for 62% of the total number of isolates obtained from these soil samples. Seven mycorrhizal morphotypes were also recovered from the Q soil, accounting for 66% of the total number of isolates from these soil samples. Five of these morphotypes (Sd1,

Sd2, Sd3, Sm1, Sm2) were found in both the E and Q soils. These shared morphotypes represented a high proportion of the mycorrhizal fungi (77% and 79% of the mycorrhizal isolates from E and Q soils, respectively). Independent of their site of origin (E or Q soils), isolates of these shared morphotypes exhibited comparable infection levels when tested in resynthesis trials. Three morphotypes (Sm3, Sm4, Sd9) were obtained exclusively from E soil and two (Sm5, Sm8) were obtained exclusively from Q soil. Isolates exclusive of the Q soil also displayed high mycorrhization capacity in *E. arborea* (Table 1).

In this study, ericoid mycorrhizal fungal diversity, measured as the number of mycorrhizal morphotypes obtained in culture, may be underestimated because unculturable ericoid fungi were not considered (Bergero et al. 2000). For example, no mycorrhizal morphotype was obtained from one of the Q plots, despite the fact that bait plants showed root infection.

Molecular analyses

ITS/RFLP profiles of mycorrhizal fungal isolates derived from the different soil samples and assigned to the same morphotype were compared. Isolates belonging to the same morphotype had identical restriction patterns, independent of their origin. When different morphotypes were compared for their ITS/RFLP profiles, at least one restriction enzyme could discriminate between isolates assigned to different morphotypes. The only exception was the Sd9 versus Sm5 comparison, where none of the three restriction enzymes produced RFLP patterns distinguishing the two morphotypes (data not shown).

ITS regions of one representative isolate from morphotypes Sd1, Sd2, Sd3, Sd9, Sm1, Sm2, Sm3, Sm5, and Sm8 were sequenced and compared. Percentage sequence identity among morphotypes ranged from approximately 64% (Sd2 versus Sm5) to 95% (Sd1 versus Sd9).

Sequences were used as queries for BLAST searches and the closest matches for each morphotype are reported in Table 2. Isolates from morphotypes Sd1, Sm5 and Sm8 were found to have their closest relatives in morphotype Sd9 (which had been previously sequenced and deposited in GenBank under the accession number AF269067, Bergero et al. 2000). These four morphotypes shared approximately 92–95% sequence identity (Sd1 versus Sm5 and Sd1 versus Sd9). The second closest match to morphotypes Sd1, Sm5, and Sm8 (first closest match to Sd9) (approximately 92–95% identity) was with a sterile mycorrhizal endophyte from the epacrid host *Astroloma pinifolium* Benth. in Australia (McLean et al. 1999). These morphotypes also shared high sequence similarity with a sterile fungus forming ectomycorrhizae with the Arctic and alpine sedge *Kobresia myosuroides* Fiori & Paoletti in Colorado (C. Schadt, personal communication). Morphotype Sm3 was found to have greatest sequence similarity with a sterile mycorrhizal endophyte from *Epacris impressa* (Epacridaceae) in Australia and

Table 1 Origin, infectiveness and morphological and cultural features (on 2% malt agar) of mycorrhizal ericoid morphotypes (*M*). Origin is based on recovery (+) or not (–) from roots of bait plants grown in soils from the *Erica arborea* (*E*) and *Quercus ilex* (*Q*) areas (+++ >75% colonized root cells, ++25–75%, + <25%)

M	Origin		Infecti-veness	Morphological and cultural features
	E	Q		
Sd1	+	+	+++	Colonies velutinous, dull cream to brown, diffusing a dark green pigment in the medium; hyphae hyaline to golden brown, sometimes monilioid; stalked brown chlamydospores, 6–8 µm diameter
Sd2	+	+	++	Colonies velutinous, grey with margins black; hyphae olivaceous brown, sometimes monilioid
Sd3	+	+	++	Colonies laccate, wrinkled, black; hyphae subhyaline to brown, monilioid
Sd9	+	–	+	Colonies waxy, wrinkled in the central area, reddish-brown with margins cream; hyphae subhyaline to golden-brown, monilioid
Sm1	+	+	++	Colonies waxy, ochraceous to salmon, wrinkled; hyphae hyaline; abundant chlamydospores in chains or in clumps
Sm2	+	+	++	Colonies waxy, wrinkled, umbonate, light cream; hyphal strands produced
Sm3	+	–	+++	Colonies waxy to velutinous, wrinkled in the central area, pink-violaceous with margins white; hyphae hyaline, often monilioid
Sm4	+	–	+++	Colonies diffuse, waxy, light cream; hyphae hyaline, few chlamidospores 10–15 µm diameter
Sm5	-	+	+++	Colonies diffuse, umbonate, grey-isabellinus; hyphae subhyaline, often monilioid
Sm8	-	+	+++	Colonies velutinous, wrinkled, white with margins incolor; hyphae hyaline

Table 2 Closest matches from BLAST searches for ITS sequences from ericoid mycorrhizal fungi. Identity (%) is given against the first sequence of the list (*E value* BLAST search E-value, *score* BLAST search score, *number* GenBank accession number, *length* length of sequenced region as bp, *M* morphotype, *S* sequences producing the most significant alignments)

M	Origin	Number	Length	S	Score	E value	Identity
Sd1	(E+Q)	AY046401	512	Sd9 (AF269067)	801	0.0	95 [491/515]
				Epacrid root endophyte AP-1 (AF099089)	733	0.0	95 [442/464]
Sd2	(E+Q)	AF269068	624	*Phialophora verrucosa* MUCL 9768 (AF050282), MUCL 9760 (AF050281), NIH8701 (U31848), NYS303 (U31846)	309	1e-81	96% [188/195]
				Capronia munkii DAOM 216390 (AF050250), DAOM 216389 (AF050249), DAOM 216388 (AF050248)	309	1e-81	96 [187/194]
				Capronia mansonii (AF050247)	309	1e-81	96 [187/194]
Sd3	(E+Q)	AY059412	560	*Botryotinia fuckeliana* BC19 (AF246943)	303	8e-80	60 [336/556]
				Monilinia urnula (Z73795), (Z73794)	303	8e-80	65 [363/559]
				Monilinia oxycocci (Z73790), (Z73789)	303	8e-80	65 [365/562]
Sm1	(E+Q)	AY046397	492	*Pezicula alba* CAL 142 (AF281377), CAL 138 (AF281376), CAL 136 (AF281375), CAL 134 (AF281374), CAL 144 (AF281373), CAL 135 (AF281372), CAL 133 (AF281371), CAL 116 (AF281370), ATCC 16504 (AF281369), DAOM 227091 (AF281368), CAL 141 (AF281367), ATCC 38338 (AF281366)	367	5e-99	91 [273/299]
				Neofabraea alba (AF141190)	367	5e-99	91 [273/299]
Sm2	(E+Q)	AY046398	512	*Guignardia philoprina* IFO 32908 (AB041243)	452	e-124	89 [372/416]
				Phialophora sp. p3901 (AF083199)	430	e-118	90 [350/386]
Sd9	(E)	AF269067	545	Epacrid root endophyte AP-1 (AF099089)	720	0.0	94 [439/463]
				Phialophora sp. p3901 (AF083199)	642	0.0	90 [507/559]
Sm3	(E)	AY046402	515	Epacrid root endophyte E2–1-6 (AF098291)	783	0.0	96 [446/463]
				Calyptrozyma arxii CBS 354.92 (AJ133432)	660	0.0	96 [412/429]
Sm5	(Q)	AY046400	514	Sd9 (AF269067)	787	0.0	94 [490/516]
				Epacrid root endophyte AP-1 (AF099089)	668	0.0	94 [425/452]
Sm8	(Q)	AY046399	517	Sd9 (AF269067)	731	0.0	94 [488/517]
				Epacrid root endophyte AP-1 (AF099089)	591	e-166	92 [431/466]

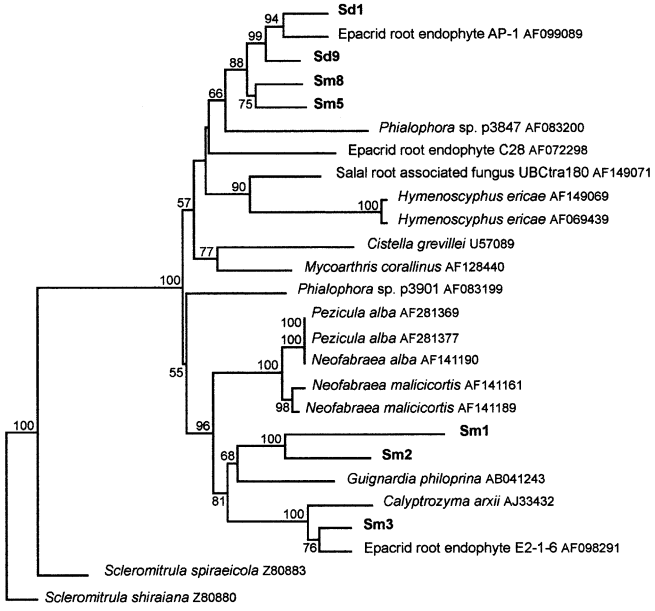

Fig. 1 Neighbour-joining tree for ITS sequences of sterile ericoid mycorrhizal morphotypes Sd1, Sd9, Sm5, Sm8, Sm1, Sm2 and Sm3 and other alignable sequences from BLAST searches. The Kimura-2-parameter model was used for pairwise distance measurement. Bootstrap values above 50% are indicated (1,000 replicates). The tree was rooted automatically

Calyptrozyma arxii Boekhout & Spaay, an ascomycete incertae sedis originally isolated from human oesophagus (Boekhout et al. 1995) (approximately 96% identity in both cases). Morphotype Sd2 showed the greatest sequence similarity to fungi of the Herpotrichiellaceae ord. Chaetothyriales class Chaetothyriomycetes [*Phialophora verrucosa* Medlar, *Capronia munkii* Unter., *C. mansonii* (Schol-Schwarz) E. Müll.; approximately 96% identity, although over a short base stretch –195 bp]. The closest matches to morphotype Sm1 were with taxa in the Dermataceae ord. Helotiales class Leotiomycetes [*Pezicula alba* E.J. Guthrie, *Neofabraea alba* (E.J. Guthrie) Verkley; approx. 91% identity], while closest matches for morphotype Sm2 were with *Guignardia philoprina* (Berk. & M.A. Curtis) Aa (strain IFO 32908; 89% identity) and an unidentified *Phialophora* species (90% identity). *Guignardia philoprina* (Berk. & M.A. Curtis) Aa (Botryosphaeriaceae, ord. Dothideales, class Dothideomycetes) is known as a weak pathogen causing leaf spot of ericaceous plants in Japan (Okane et al. 2001); strain IFO 32908 was isolated from leaf litter of *Rhododendron* sp. in The Netherlands. Morphotype Sd3 had its closest relatives in taxa in Sclerotiniaceae ord. Helotiales class Leotiomycetes [*Botryotinia fuckeliana* (de Bary) Whetzel, *Monilinia urnula* (Weinm.) Whetzel, *M. oxycocci* (Woronin) Honey] but sequence identity was rather low (60–65% identity).

Sequences of morphotypes Sd1, Sd9, Sm5, Sm8, Sm1, Sm2 and Sm3 were subjected, along with other alignable sequences from GenBank, to a Neighbour-Joining (NJ) analysis (Fig. 1). Automatic root location positioned the root at the *Scleromitrula* node. Within the ingroup, sequences of morphotypes Sd1, Sd9, Sm5 and Sm8 clustered together and with the epacrid endophyte from *A. pinifolium* (88% bootstrap value) within a larger group (although with low bootstrap support) also including sequences from sterile ericoid mycorrhizal fungi from salal (AF149071) and *Woollsia pungens* F. Muell. (AF072298), the unidentified *Phialophora* species p3847 (AF083200), *Hymenoscyphus ericae* (D.J. Read) Korf & Kernan (AF149069, AF069439), *Cistella grevillei* (Berk.) Raitv. (U57089) and *Mycoarthris corallinus*, a putative new arthroconidial fungus of helotialean affinities (AF128440). A well-supported group (81% bootstrap value) comprised sequences from morphotypes Sm1, Sm2 and Sm3 as well as sequences of the epacrid symbiont E2–1-6 (AF098291), *C. arxii* (AJ133432) and *G. philoprina* (AB041243). Within this complex, Sm1 and Sm2 clustered together with 100% bootstrap support and with *G. philoprina* (68% bootstrap), while Sm3 clustered with E2–1-6 and *C. arxii* (100% bootstrap).

Discussion

In the Mediterranean ecosystem investigated, soil samples from the pure *Quercus ilex* forest not only maintained an effective ericoid mycorrhizal inoculum, but also displayed high ericoid fungal diversity, despite the absence of the ericaceous host plant. There was a wide overlap in ericoid morphotype composition in the two plant communities, with five mycorrhizal morphotypes present in both the Q and E areas, which made up a high proportion of the mycorrhizal isolates.

When ITS sequences from the ericoid morphotypes were compared to sequences in GenBank, none displayed close relatedness to *Hymenoscyphus ericae* or *Oidiodendron* spp., the dominant taxa in the diverse assemblages of symbionts colonizing the ericaceous plants investigated to date (Hambleton and Currah 1997; Perotto et al. 2002; Sharples et al. 2000). Percentage similarities with sequences from the BLAST searches were moderate to low, and this also may have influenced the outcome of the NJ analysis (since similarities were generally rather low, some clustering in the NJ tree may be an artefact of the relatively limited range of sequences available for comparison). *Erica arborea* fungal symbionts, therefore, likely represent taxa having no close relative among fungi currently included in GenBank. This is probably also the case for sterile isolates from other ericaceous hosts (Perotto et al. 2002). Isolates from *E. arborea* in several cases (morphotype Sm3 and morphotype complex Sd1-Sd9-Sm5-Sm8) were related more closely to isolates from epacridaceous than ericaceous hosts. As regards affinities with known taxa, there is evidence that the spectrum of *E. arborea* mycobionts spans different classes within Ascomycota (e.g. Leotiomycetes and Dothideomycetes). Such a spectrum is dominated, however, by the complex of morphotypes Sd1, Sd9, Sm5 and Sm8, which appears to be a unique aggregate having no

named representatives in GenBank but with likely affinities in Helotiales (Leotiomycetes).

In an investigation carried out on undisturbed and disturbed sites with sparse epacrid understorey in Australian jarrah forests, close proximity of suitable host plants was found to be a key determinant of soil infectivity (Hutton et al. 1997). In contrast, our results indicate that viable inocula of ericoid mycorrhizal fungi may persist in the absence of a living host plant. A similar result was obtained in a study on mycorrhizae of *Pernettya macrostigma*, an endemic member of Ericaceae in New Zealand. A pot experiment with soil from native forests lacking heaths and epacrids provided evidence of the presence of mycorrhizal inoculum (Brook 1952). Similarly, Pearson and Read (1973) found that moorland soils with no ericaceous vegetation may contain fungi able to form mycorrhiza in *Calluna vulgaris*.

Several hypotheses can be put forward to explain such persistence. There may be invasion arising from fungal propagules occurring in the areas where *E. arborea* is present. However, the lack of spore production by most ericoid fungi (Hutton et al. 1994; Perotto et al. 1996; Hambleton and Currah 1997; Bergero et al. 2000) and specifically by our ericoid fungi (as assessed in culture) may limit considerably species diffusion and, therefore, invasion. Furthermore, sporadic patches where cutting had allowed reappearance of *E. arborea* were scarce within the locality studied, which was an almost pure *Q. ilex* forest.

Secondly, ericoid fungi may be maintained in the soil thanks to their free-living, saprotrophic growth. Given the general ability of ericoid mycorrhizal fungi to grow in pure culture on standard media, and to degrade complex organic substrates such as cellulose, chitin, pectin, proteins and lignin (Pearson and Read 1975; Haselwandter et al. 1990; Varma and Bonfante 1994; Kerley and Read 1995; Perotto et al. 1997), they may compete successfully with saprotrophic and ectomycorrhizal fungi for dead organic substrates. High enzymatic competence, slow growth, apparent absence of reproductive structures and persistence in habitats experiencing long, hot, dry seasons, define ericoid fungi as stress-tolerant fungal species sensu Grime (1977), well suited to survive under the stressful conditions of Mediterranean summers.

A third possibility derives from the results of a previous study in the same Mediterranean forest demonstrating that some ericoid fungi can tightly associate with *Q. ilex* ectomycorrhizal roots (Bergero et al. 2000). Association of ericoid fungi with ectomycorrhizal roots either as true mycorrhizal partners or as root associates (as suggested by Bergero et al. 2000) may make these fungi more tolerant of the Mediterranean summer drought. Villeneuve et al. (1989) found that saprotrophic fungi declined from 125 to six species along a gradient of increasing environmental stress, whereas species richness of ectomycorrhizal fungi was less affected (from 35 to 28 along the same stress gradient). The ectomycorrhizal plant host could, therefore, buffer environmental stresses and guarantee persistence of ericoid fungi in soils lacking suitable host plants.

In conclusion, a diverse assemblage of ericoid fungi can persist and maintain mycorrhization ability in soil in the absence of the host plant. This may be of great ecological importance in Mediterranean ecosystems, for example by assuring ex novo mycorrhization of *E. arborea* seedlings in *Q. ilex* pure stands after disturbance.

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